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EFFECT OF CADMIUM, LEAD AND NICKEL ON IMBIBITION, WATER UPTAKE AND GERMINATION FOR THE SEEDS OF DIFFERENT PLANTS

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ABSTRACT

The effect of different metal salts on the imbibition, water uptake and germination of *Phaseolus vulgaris* and *Rumex scutatus* was studied under laboratory conditions. Seeds were treated with solutions of the following concentrations; 0, 100, 1000, 10000 mg L^{-1} Pb, 0, 50, 100, 1000 mg L^{-1} Ni and 0, 80, 160, 320 mg $L^{-}1$ Cd. Lead and cadmium was delayed germination in *P.vulgaris*.Germination was strongley inhibited by higher Ni and Pb^{+2} concentrations. However, germination rate was significantly greater at 100 mg L^{-1} Pb than lower concentrations of other metal salts. It was also shown that imbibiton of seeds was affected by metal salts.

Key Words: *Cadmium, germination, imbibition, lead, nickel, Phaseolus vulgaris, Rumex scutatusl.*

FARKLI BİTKİ TOHUMLARININ ÇİMLENME, İMBİBİSYON VE SU ALINIMINA KADMİYUM, KURŞUN VE NİKELİN ETKİSİ

ÖZET

Phaseolus vulgaris ve *Rumex scutatus* tohumlarında imbibisyon, su alınımı ve çimlenmeye farklı metal tuzlarının etkisi laboratuar koşullarında incelenmiştir. Tohumlar, değişen konsantrasyonlarda; 0, 100, 1000, 10000 mg L^{-1} Pb, 0, 50, 100, 1000 mg L^{-1} Ni ve 0, 80, 160, 320 mg $L^{-}1$ Cd metal solusyonları ile muamele edilmiştir. Kurşun ve kadmiyum *P.vulgaris*'de çimlenmeyi geciktirmiştir. Çimlenme yüksek Ni ve Pb $^{+2}$ konsantrasyonunda inhibe olmuştur. Bununla birlikte, çimlenme oranının diğer metallerin düşük konsantrasyonlarından ziyade, 100 mg L^{-1} Pb'de belirgin şekilde daha yüksek olduğu tespit edilmiştir. Ayrıca, tohumlardaki imbibisyonun da metal tuzlarından etkilendiği gözlenmiştir.

Anahtar Kelimeler: *Kadmiyum, çimlenme, imbibisyon, kurşun, nikel, Phaseolus vulgaris, Rumex scutatus*

1. INTRODUCTION

Soil pollution by different kinds of heavy metals has become a critical environmental concern due to its potential adverse ecological effects. Although, heavy metals occur naturally at low concentrations in soil systems, they are considered as soil contaminants because of their acute and chronic toxicity. Some of them such as lead and cadmium are special concern due to its relatively high mobility in soils [1]. Plants are an important link in the pathway by which excessive amounts of heavy metals are channeled into the food chain and biological cycles [1]. Germination is one of the most common and effective processes for improving the quality of cultivated plants which are widely consumed all around the word. The process is influenced by external factors such as germination time, presence or absence of light, mineral or metal composition of soil, all of which aid or inhibit the germination process in relation to the reserve materials of the seed [2].

The aim of the presented study was to investigate the effect of different heavy metal solutions on seed imbibition, water uptake and germination activity for *P.vulgaris* and *R.scutatus*. In choosing species for investigation it was decided to concentrate on plants of economic value. *Rumex scutatus* which was just started to be cultivated for salad dressing purposes and *Phaseolus vulgaris* are important grain in many countries. Legumes contain a high concentration of proteins, carbohydrates and dietary fiber and make an important contribution to human diet in many countries [3].

2. MATERIAL AND METHODS

2.1. Test Chemicals

Cadmium nitrate (Cd $(NO_3)_2 4H_2O$), nickel nitrate (Ni $SO_4 6H_2O$) and lead nitrate (Pb $(NO_3)_2$) was used without further purification. Preliminary tests were performed to determine the appropriate sensitivity range for seeds of test plants. The exposure concentrations for both test plants were 0, 80, 160 and 320 mg Cd L^{-1} from Cd $(NO_3)_2 4H_2O$; 0, 100, 1000 and 10000 mg Pb L^{-1} from $PbCl_2$ and 0, 50, 100 and 1000 mg Ni L^{-1} from (Ni $(NO_3)_2 6H_2O$). Stock solutions of cadmium, nickel and lead were prepared in distilled water at desired concentrations prior to using. Furthermore, stock solutions were diluted with distilled water to obtain suitable concentrations of test chemicals. Double distilled water was used as the control. Imbibition, water uptake and germination responses of the plants exposed to a range of cadmium, nickel and lead were recorded.

2.2. Imbibition of Seeds in Metal Salts and Water Uptake by Germinating Seeds

Seeds of plants were supplied by the local Ministry of Agriculture office located in Kayseri, Turkey. Damaged or empty seeds hulls were discarded. The imbibition and germination experiments described below were conducted 4–6 months after seed collection.

To determine the effect of metal salts on imbibition, seeds were treated with metal salts (these are; Pb $(NO_3)_2$, (Ni $(NO_3)_2 6H_2O$) and Cd $(NO_3)_2 4H_2O$) during the imbibition period. Fifty seeds from each plant for each treatment were placed in different glass beakers containing 100 ml of a solution containing each metal from salts at different concentrations and seeds in 100 ml of deionized water were used as a control [4]. Each test was carried out in five replicates. Imbibition was ended just before the radicle pierced the seed coat. All of the experiments were carried out in a temperature and light controlled growth cabinet at a temperature of 25 ^{0}C which is optimal germination temperature obtained from pre-germination study for seeds of both plants.

An experiment was also designed to test the influence of different kind of metals salts on the ability of seed germination. For this reason, the weight of seeds during imbibitions was determined basing on the seeds of *Phaseolus vulgaris* and *Rumex scutatus*. The seed samples were weighed during the imbibition period at appropriate time intervals such as every 5 hours. Treatment with different kinds of metal was ended just before the radicle pierced the seed coat.

After the imbibition, the seeds of plants in solutions of different kind of metals were washed in deionized water and then sown on moist filter paper and transferred the Petri dishes. Germination was carried out in the plant growth cabinet. The growth cabinet conditions were minimum temperature: 15 ^{0}C, maximum temperature: 30 ^{0}C (resembling the summertime temperature regime in the field), with a 14 h of daylight and 10 h of darkness photoperiod.

Germination was recorded daily for 20 days or until no germinating seeds were observed for more than five days. No viability test was performed to ungerminated seeds after the experiments ended, since most of them showed clear signs of rotting and presumably were not viable. Thus, final germination percentages were taken as direct measure of seed viability.

The SPSS (Statistical Package for The Social Sciences) statistical program was used to calculate standard errors, mean and variance. Analysis of variance (ANOVA) was used to determine if significant differences were present among means.

3. RESULTS AND DISCUSSION

3.1. The Effect of Metal Salts on Imbibition and Water Uptake by Germinating Seeds

In the tested concentration range, a slight effect of nickel and cadmium on water uptake by seeds was found. According to results, significant reduction of water uptake by seeds was observed at 50 mgL^{-1} Ni and 80 mgL^{-1} Cd Concentrations (Figure 1-2). In addition, statistically significant reduction in water uptake during the imbibition was obtained from 10000 mgL^{-1} Pb^{+2} and 1000 mgL^{-1} Ni solutions. Cadmium in a concentration of 160 mgL^{-1} did not restrict water uptake in *Rumex scutatus*. These results indicate that the restriction of water uptake by seeds during the imbibition could not have been an important cause of the delay in germination by cadmium for *P.vulgaris* and *R.scutatus*. Similar results obtained from *P.vulgaris* by Wierzbicka and Obidzinska [4]. Levitt [5], reported that the concentration of many metal salts which osmotic salt stress can occur is 10^{-3} M and it can create an osmotic potential to prevent water uptake or can provide conditions for the entry of ions that may be toxic to the embryo. Results obtained from present study supported these hypothesis, as it had been shown, salts of metals caused lower water uptake and its transport in *P.vulgaris* and *R.scutatus*. In addition, the results of this study show that the inhibition of germination result from metals at high concentrations was not the result of a reduction of water uptake, however the toxic effect of metal salts. These results were supported by other authors such as Fargasová [6].

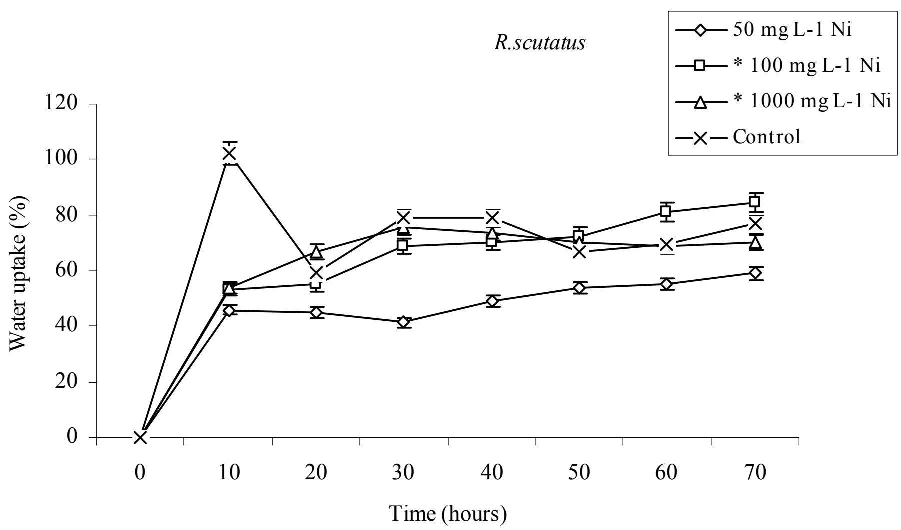

Figure 1-a: Water uptake during 70 h of imbibiton in three (50 , 100 and 1000 mg L^{-1} Ni) different concentrations of nickel solutions with control groups. * Statistically significant difference from the control curve ($p< 0.001$).

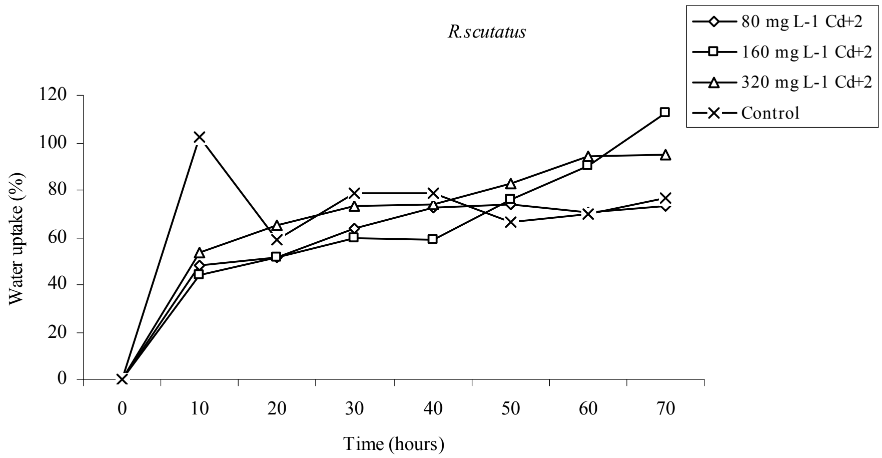

Figure 1-b: Water uptake during 70 h of imbibiton in three (80 , 160 and 320 mg L^{-1} Cd^{+2}) different concentrations of cadmium solutions with control groups.

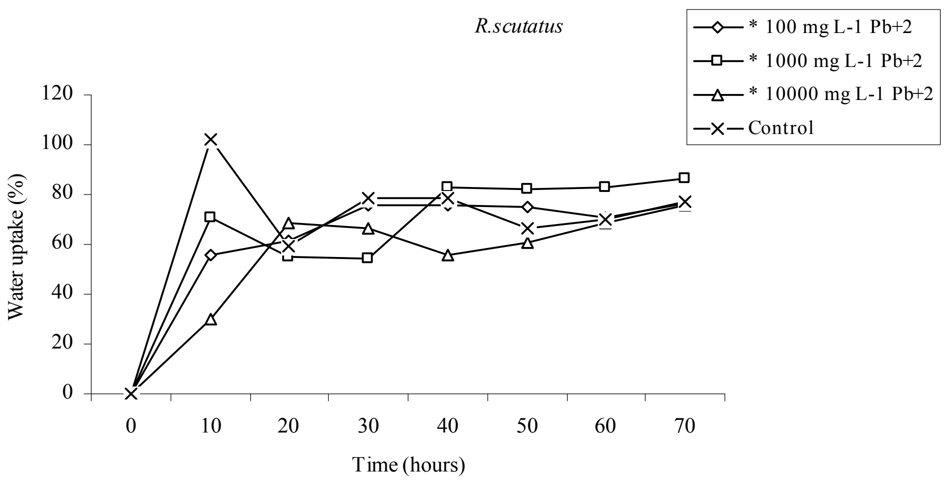

Figure 1-c: Water uptake during 70 h of imbibiton in three (100 , 1000 and 10000 mg L^{-1} Pb^{+2}) different concentrations of lead solutions with control groups. * Statistically significant difference from the control curve ($p< 0.001$).

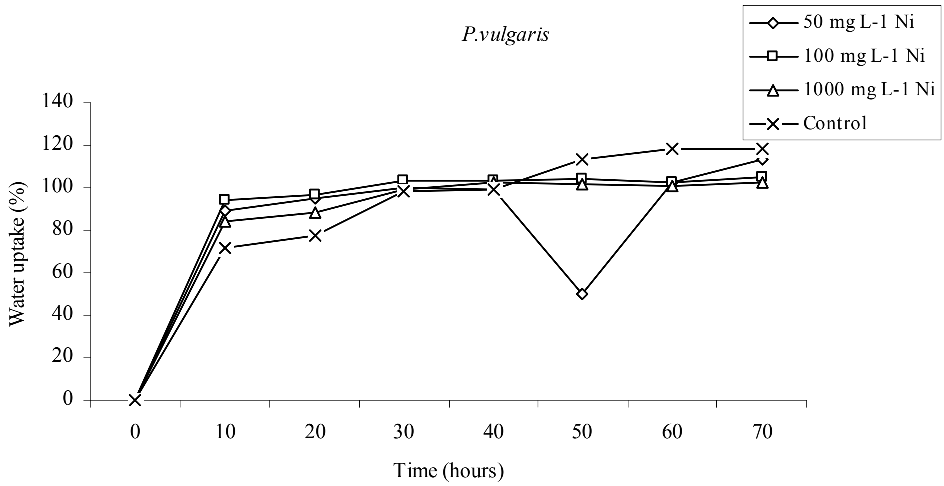

Figure 2-a: Water uptake during 70 h of imbibiton in three (50 , 100 and 1000 mg L^{-1} Ni) different concentrations of nickel solutions with control groups.

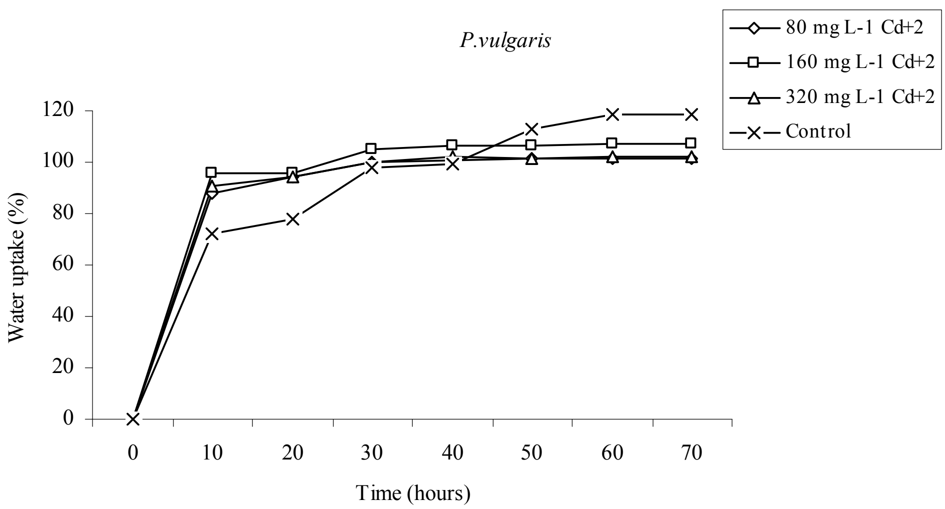

Figure 2-b: Water uptake during 70 h of imbibiton in three (80 , 160 and 320 mg L^{-1} Cd^{+2}) different concentrations of cadmium solutions with control groups.

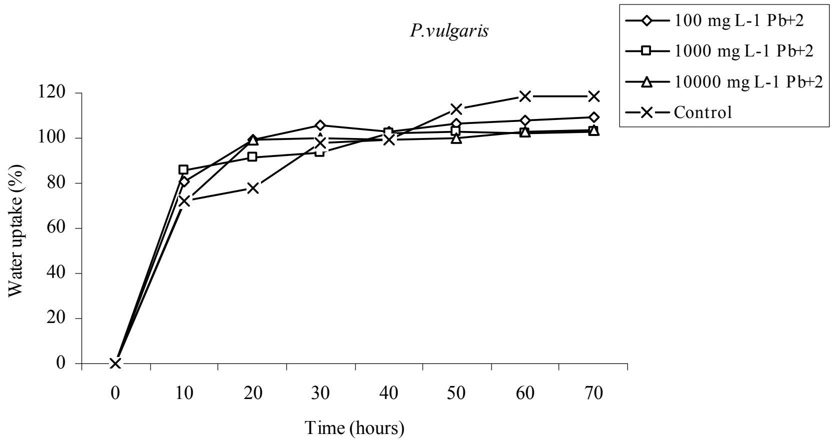

Figure 2-c: Water uptake during 70 h of imbibiton in three (100 , 1000 and 10000 mg L^{-1} Pb^{+2}) different concentrations of lead solutions with control groups.

3.2. Germination

Seed germination is a critical phase of the life-cycle in most plant species, particularly in cultivar plants. Stressful conditions exert a strong effect on the dynamics of seed germination and seedling establishment, which are in turn driving forces of population dynamics [3,7]. Present results showed that the highest element concentrations caused statistically significant inhibition of seed germination (Figure 3-4), for example, the lowest germination rate (0 %) was obtained at 10000 mgL^{-1} lead concentrations and the highest germination rate (40 %) was obtained at 100 mgL^{-1} lead concentrations, this rate decreased to 36 % at 1000 mgL^{-1} Pb for *R.scutatus*. Similarly, the lowest germination percent (14 %) was obtained at 320 mgL^{-1} cadmium and the highest germination percent (21 %) was obtained at 80 mgL^{-1} nickel for *Phaseolus vulgaris*. Similar results obtained from different concentrations of Ni for both plants. Same trend have been observed Wierzbicka and Obidzinska [4], who showed concentrations that the highest concentration of lead causes inhibition of germination of different kind of seeds such as *Pisum sativum*. However, same authors implied that, the amount of lead per seed mass unit was important; it means that the concentration of lead is not important on germination rate. This issue may relate to permeability of seed coat to metal ions. Due to the different degree of permeability of seed coats to metals such as lead led to a different degree of germination inhibition. Seeds of *Phaseolus vulgaris* may lose their germinability in different concentrations of metals because it has highly lead permeable seed coats.

If the seed coat is permeable to metals, it can cause delaying or inhibition of germination. As a result, the results presented in this study show that germination was delayed in seeds of *P.vulgaris* due to exposition to metals especially lead and nickel (Figure 4). In contrast, according to Wierzbicka and Obidzinska [4], lead did not have a significant effect on germination in *Rumex thyrsiphlorus*.

The results can support to the observations described by other authors on the effect of different metals on seed germination. For example, Wozny et al. [8], showed that *Lupinus luteus* seeds lost some degree of the germinatinability when expose to lead. Same trend have been observed in *Orzya sativa* by Mukherji and Maitra [9] and in *Sinapis alba* by Fargasova [6].

Percent germination of *Rumex scutatus* and *Phaseolus vulgaris* was significantly decreased at 1000 mgL^{-1} Ni and 10000 mgL^{-1} Pb concentrations (Figure 3-4). Results indicated that seed germination was resistant to Cd toxicity at most levels used for *Rumex scutatus*. Similar results obtained from seeds of different kind of plants for example, *Cucumis sativus* and *Zea mays* by An [11].

A one-way ANOVA of the water uptake during the imbibition at each metal salt solutions revealed that some of them significantly effected to water uptake, such as; the various metal salt concentrations had a significant effect on water uptake of seeds of *R. scutatus*. (for nickel, $F= 1.85$; $df=3$; $P<0.001$; for cadmium, $F= 2.86$; $df=3$; $P<0.005$; for lead, $F= 0.393$; $df=3$; $P<0.001$). Similiarly, different metal salts effected to water uptake of seeds of *P.vulgaris* (for nickel, $F= 0.081$; $df=3$; $P<0.005$; for cadmium, $F= 0.177$; $df=3$; $P<0.005$; for lead, $F= 0.70$; $df=3$; $P<0.001$). Furthermore, the germination rate at each metal salt solutions revealed that effected to germination of seeds for example; in *R.scutatus* ; for nickel, F= 1.485; df=2; P<0.005; for cadmium, F= 1.532; df=2; for lead, F= 6.89; df=2; P<0.001, in *P.vulgaris*; for nickel, F= 1.327; df=2; P<0.005.

In conclusion, it can be said that metal salt stress can act on seeds in two ways; first is the prevention of water uptake by seeds, second is providing conditions for the entry of metal ions that may be toxic to the embryo.

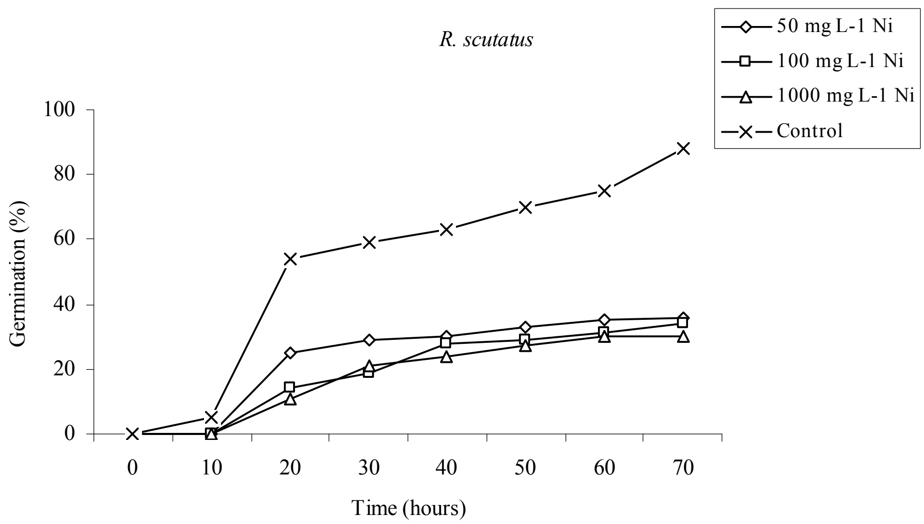

Figure 3-a: Germination percent of seeds during 70 h in three (50 , 100 and 1000 mg L^{-1} Ni) different concentrations of nickel solutions with control groups.

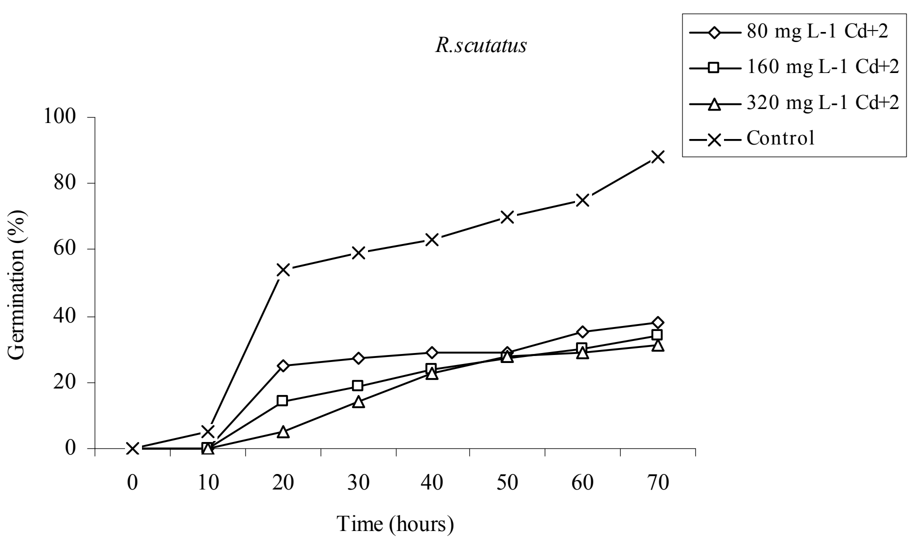

Figure3-b: Germination percent of seeds during 70 h in three (80 , 160 and 320 mg L^{-1} Cd^{+2}) different concentrations of cadmium solutions with control groups.

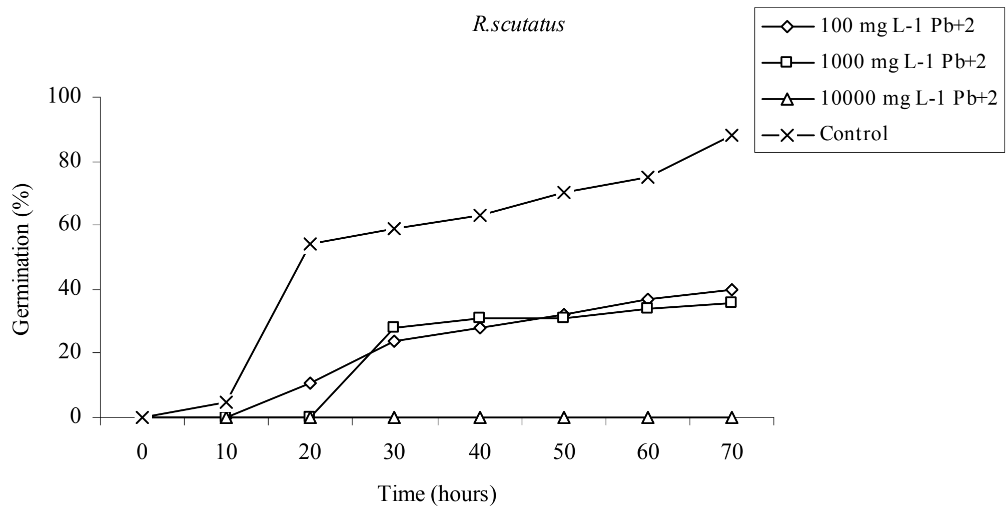

Figure 3-c: Germination percent of seeds during 70 h of imbibiton in three (100 , 1000 and 10000 mg L^{-1} Pb^{+2}) different concentrations of lead solutions with control groups.

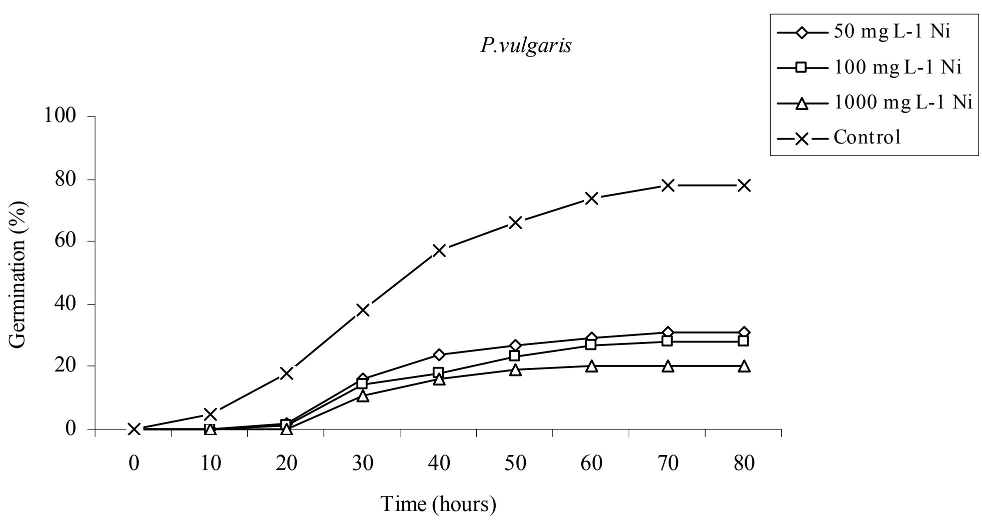

Figure 4-a: Germination percent of seeds during 70 h in three (50 , 100 and 1000 mg L^{-1} Ni) different concentrations of nickel solutions with control groups.

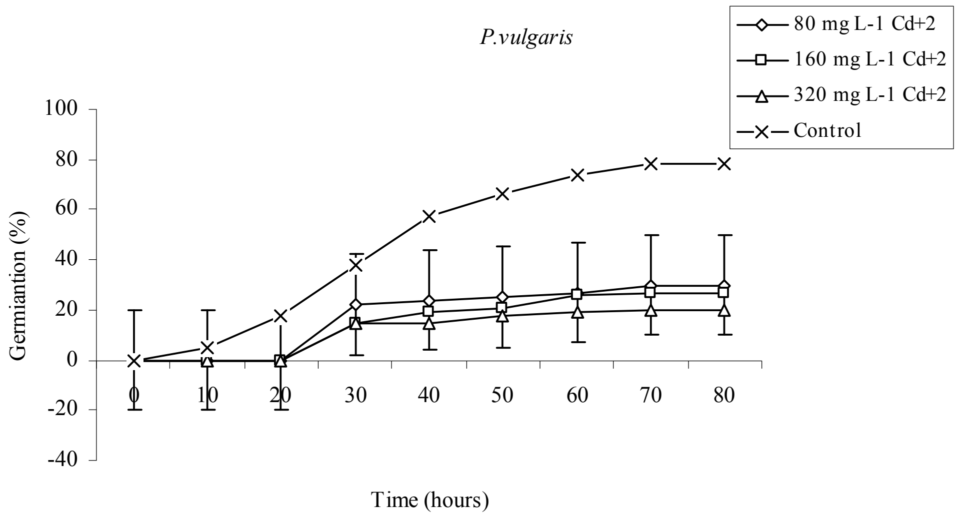

Figure 4-b: Germination percent of seeds during 70 h in three (80 , 160 and 320 mg L^{-1} Cd^{+2}) different concentrations of cadmium solutions with control groups.

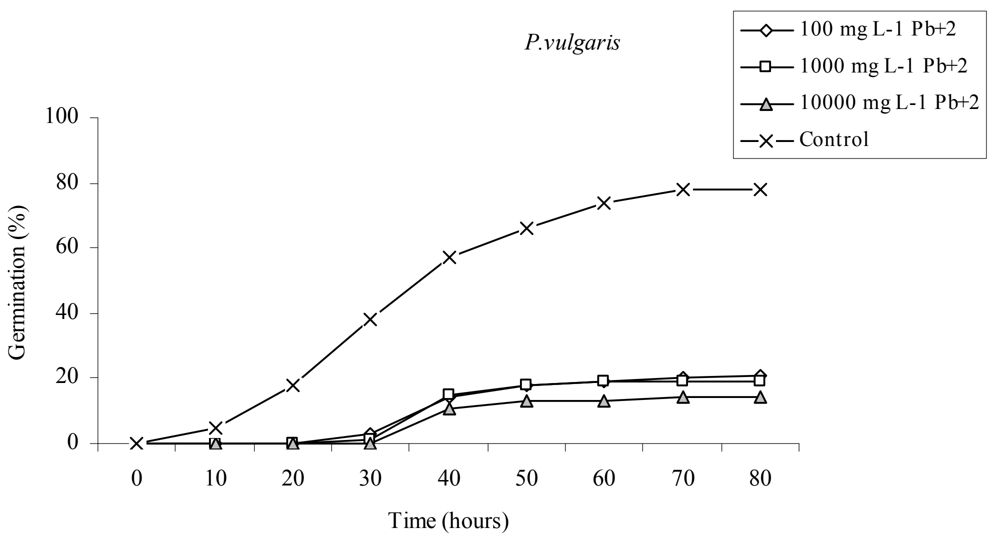

Figure 4-c: Germination percent of seeds during 70 h of imbibiton in three (100 , 1000 and 10000 mg L^{-1} Pb^{+2}) different concentrations of lead solutions with control groups.

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